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New cytotoxic phloroglucinols, baeckenones D–F, from the leaves of Indonesian *Baeckea frutescens*

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ABSTRACT

Three new phloroglucinols, baeckenones D–F (**1**–**3**), were obtained from the leaves of Indonesian *Baeckea frutescens*, along with the known unusual *endo*peroxide, phloroglucinol (**4**). The structures of the isolated compounds were elucidated by 1D and 2D NMR and HREIMS spectra. Furthermore, the stereochemistry of baeckenone D (**1**) was established by an X-ray diffraction analysis. Among the isolated compounds **1**–**4**, baeckenone F (**3**) showed moderate cytotoxic activities against human pancreatic (PSN-1), lung (A549), and breast (MDA-MB-231) cancer cell lines, with IC_{50} values of 33.3 μM, 34 μM, and 39.3 μM, respectively.

1. Introduction

Phloroglucinol-type secondary metabolites, derived from the plants of the family Myrtaceae, reportedly possess a wide range of biological activities, including antibacterial, antifungal, antiplasmodial, antimalarial, antitumor, and immunomodulatory effects [1–7]. As examples, sideroxylonal B, macrocarpal A, eucalyptal D, and eucalyptal E exhibited significant cytotoxic activities against various human cancer cell lines [6–7]. Thus, we have performed further investigations of the chemical constituents of the plants belonging to the family Myrtaceae, toward the discovery of new drug leads.

Baeckea frutescens L is a medicinal plant belonging to the family Myrtaceae. This plant has been used as a source of traditional medicine for the treatment of various human diseases, such as influenza, rheumatism, fever, headache, and abdominal pain. Previous studies of this plant reported anti-inflammatory, antipyretic, antidysenteric, antibacterial, and cytotoxic activities [8–14]. Furthermore, chemical investigations of this plant have led to the isolation of terpenoids, flavonoids, and phloroglucinols [15–20]. Recently, we have reported the isolation and structure determination of several acylphloroglucinols (baeckenones A–C), and found that baeckenone B exhibits good growth inhibitory activity against the Gram-positive bacterium *Bacillus subtilis*, with an MIC of 40 μM [21]. In the course of our search for new bioactive metabolites from *B. frutescens*, we isolated three new cytotoxic phloroglucinol derivatives (**1**–**3**), along with a known phloroglucinol (**4**) (Fig. 1). We now describe the isolation, structure elucidation, and cytotoxic activities of the derivatives against human pancreatic (PSN-1), lung (A549), and breast (MDA-MB-231) cancer cell lines. (Table 2.)

2. Experimental

2.1. General experimental procedures

IR spectra were measured using a JASCO IR-460 plus spectrophotometer (Japan Spectroscopic Co, Ltd., Tokyo, Japan). Optical rotations were determined using a JASCO DIP-140 digital polarimeter. UV–Vis spectra were obtained on a Nano Drop 2000c spectrophotometer (Thermo Scientific, Waltham, MA, USA). NMR spectra were recorded on a Varian UNITY 600 MHz spectrometer in $CDCl_3$, with TMS (Wako Pure Chemical Industries, Ltd., Osaka, Japan) as an internal standard. The HREIMS data were measured on a JEOL MStation JMS-700 spectrometer (JEOL Ltd., Tokyo, Japan). Medium Pressure Liquid Chromatography (MPLC) was performed on a Büchi Sepacore system (Büchi Labortechnik AG, Flawil, Switzerland) with silica gel (60 N, spherical, neutral, 40–50 μm, Kanto Chemical Co., Inc., Tokyo, Japan) and Cosmosil $75C_{18}$-OPN (Nacalai Tesque Inc., Kyoto, Japan). Normal phase preparative HPLC was performed on a Waters Delta 600 system, with a Cosmosil

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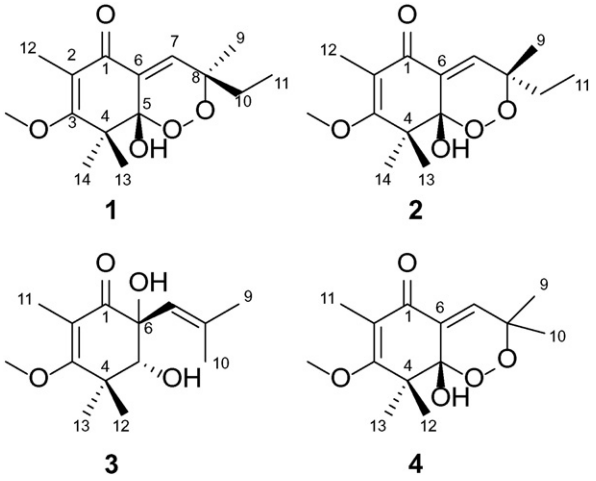

Fig. 1. The structures of compounds **1**–**4**.

5SL-II column (10 × 250 mm, 5 μm; flow rate 2.0 mL min^{-1}), a 600 F pump, and a 2998 Photodiode array detector, with monitoring at 210 nm. TLC was performed on precoated silica gel 60F_{254} plates (0.25 or 0.50 mm thickness, Merck KGaA, Darmstadt, Germany). Preparative TLC was performed in vertical type rectangular chambers (Yazawa, Tokyo, Japan), under conditions saturated with the developing solvent. The cell lines, PSN-1 (human pancreatic cancer), A549 (human lung cancer), and MDA-MB-231 (human breast cancer), were available and maintained in our laboratory. Cell culture flasks and 96-well plates were purchased from Corning Inc. (Corning, NY, USA). An SH-1200 microplate reader (Corona Electric Co., Ltd., Hitachinaka, Japan) was used to measure the absorbance of the cells in the cytotoxic activity assay.

2.2. Plant material

The leaves of *B. frutescens* were purchased in the Pasar Gedhe Market (Solo, Indonesia), and identified by Saifudin Azis, Ph.D. (School of Pharmacy, Universitas Muhammadiyah Surakarta, Indonesia). The voucher specimen (28298) was deposited at the Museum of Materia Medica, Analytical Research Center for Ethnomedicines, Institute of Natural Medicine, University of Toyama, Toyama, Japan.

2.3. Extraction and isolation

The air-dried and powdered leaves of *B. frutescens* (425 g) were exhaustively extracted with $CHCl_3$ (3 × 1.5 L) in an ultrasonic bath, for 90 min each at room temperature. The resulting solution was evaporated under reduced pressure to yield the $CHCl_3$ extract (41 g). The concentrated extract was separated by normal phase MPLC (1.85 kg; 40–50 μm; 100 × 460 mm; flow rate = 25 mL min^{-1}), eluted with *n*-hexane–EtOAc by gradually increasing the polarity system (from 1:0 to 0:1), to give ten fractions (Fr. 1–10). Fr. 3 (1.6 g) was separated by reversed phase MPLC (120 g; 36 × 160 mm), eluted with an isocratic mobile phase (MeCN–MeOH–H_2O = 4:3:3, *v*/v), to furnish seven subfractions (Fr. 3.1–3.7). Subfraction Fr. 3.1 (60 mg) was further subjected to SiO_2 preparative TLC (200 × 200 mm, 185 mm separation distance, *n*-hexane–EtOAc = 1:1), to afford **3** (1.3 mg), **4** (20 mg), and a mixture of compounds **1** and **2**. The mixture of **1** and **2** was then purified by normal phase HPLC (*n*-hexane–EtOAc = 50:50, *v*/v; flow rate = 2.0 mL min^{-1}; detection UV 254 nm), to provide compounds **1** (10 mg) and **2** (1.5 mg). The structures of all compounds were elucidated using 1D and 2D NMR, and MS spectra, and compared with published data.

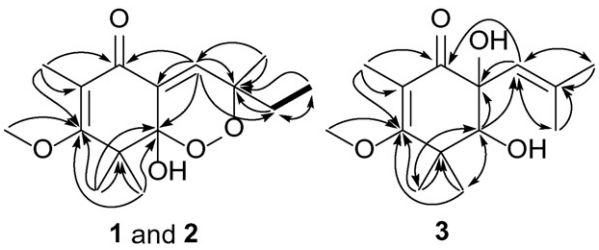

Fig. 2. ^1H–^1H COSY (bold lines) and key HMBC (arrows) correlations of **1**–**3**.

Baeckenone D (**1**): Colorless needles; MP 121–123 °C; $[\alpha]_D^{25}$ 0 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) nm: 286 (3.82); IR (KBr) ν_{max} 3395, 2975, 1670, 1604, 1459 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data (see Table 1); HREIMS: *m/z* 282.1477 $[M]^+$ (calcd. for $C_{15}H_{22}O_5$, 282.1467).

Baeckenone E (**2**): White powder; $[\alpha]_D^{25}$ 0 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) nm: 281 (3.83); IR (KBr) ν_{max} 3395, 2925, 1670, 1604, 1460 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data (see Table 1); HREIMS: *m/z* 28–2.1459 $[M]^+$ (calcd. For $C_{15}H_{22}O_5$, 282.1467).

Baeckenone F (**3**): Yellow amorphous mass; $[\alpha]_D^{25}$ − 44 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) nm: 259 (3.51); IR (KBr) ν_{max} 3444, 1668 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data (see Table 1); HREIMS: *m/z* 254.1528 $[M]^+$ (calcd. for $C_{14}H_{22}O_4$, 254.1518).

2.4. X-ray crystallographic analysis of baeckenone A (*1*)

X-ray data for compound **1** were collected on a Bruker APEX 2 CCD area detector diffractometer with a Helios multi-layered confocal mirror ($\phi - \omega$ scans), using Mo *Kα* radiation (λ = 0.71069 Å) from a Bruker TXS fine-focus rotating anode. The Bruker *APEX 2* software was used for cell refinement and data reduction. The programs *SHELXS*-97, *SHELXL*-2014, and *PLATON* were used for the structure solution, the structure refinement, and the ORTEP plot, respectively [22–24]. Crystallographic data for the structure of compound **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 1441723). Copies of these data can be obtained, free of charge, upon application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223–336,033 or e-mail: deposit@ccdc.cam.ac.uk).

Crystal data of baeckenone D (**1**): $C_{15}H_{22}O_5$, *M* = 282.32, orthorhombic, space group *Pbca*, *a* = 10.5445(15) Å, *b* = 10.4510(15) Å, *c* = 27.954(4) Å, *V* = 3080.6(8) $Å^3$, *Z* = 8, D_{calcd} = 1.217 g/cm^3, *T* =

Table 1
^1H (600 MHz) and ^{13}C NMR (150 MHz) data of **1**–**3** in $CDCl_3$ (δ in ppm)[a].

No.	**1**		**2**		**3**	
	δ_C	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)
1	186.1		186.4		201.1	
2	117.4		117.6		114.4	
3	178.2		178.4		179.6	
4	45.8		46.0		43.1	
5	98.5		98.6		79.6	3.63 (s)
6	133.0		132.6		78.7	
7	139.1	6.94 (s)	139.8	6.98 (s)	121.2	5.33 (s)
8	81.4		81.5		138.2	
9	21.6	1.42 (s)	20.3	1.34 (s)	19.3	1.71 (s)
10	30.5	1.69 (q, 7.6)	29.3	1.72 (m)	28.2	1.73 (s)
11	7.8	0.95 (t, 7.6)	8.1	0.99 (t)	10.7	1.90 (s)
12	10.1	1.90 (s)	10.3	1.90 (m)	18.9	1.25 (s)
13	16.5	1.35 (s)	16.7	1.27 (s)	26.9	1.28 (s)
14	24.9	1.17 (s)	25.1	1.15 (s)		
3-OCH_3	61.9	3.92 (s)	62.1	3.92 (s)	62.1	3.91 (s)
5-OH		3.24 (s)		3.33 (s)		

[a] The assignments were based on HMQC, HMBC, and NOESY experiments.